Disorders of lipid metabolism & atherosclerosis

Lectures from molecular medicine school year 2009/2010
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Introduction

- Atherosclerosis is a medical problem only from the XIX\textsuperscript{th} century
  - Short lifespan due to other diseases
  - No clinical biochemistry
- Rokitansky (Vienna, 1852) incrustation [thrombogen] theory
- Virchow (Berlin, 1855) infiltration [lipid] theory
- Framingham study (1948)
- 2000 \(\approx\) 25 theories and numeral epidemiological studies
Overview

- General pathological physiology
  - Blood lipids and lipoproteins (function & metabolism)
  - Lipids & atherosclerosis (epidemiological & experimental proofs)
  - Other factors of atherosclerosis development
  - Comments on diagnostics
  - Classification of dyslipoproteinemias
  - Genetic background of lipid metabolism disorders and atherosclerosis

Special pathological physiology – tissue and organ level, CHD, stroke

Cholesterol, free and esters with fatty acids

- 275 mmol in the body
  - 50 mmol LP, GIT, liver
  - 25 mmol fat tissue
  - 90 mmol muscles & vessel wall
  - 110 mmol nervous system

- 3 mmol/d exchange

27.10.09
**Catch 22**

- **Cholesterol**
  - In: food and synthesis in cells (from CoA*)
  - Breakdown: no
  - Out: bile (enterohepatal circuit); stool
- **LDL cholesterol**
  - The normal level (3.1 – 3.9 mmol/l) is not normal for the endothel
  - Newborns & atherosclerosis resistant
  - animals only \( \approx 0.8 \) mmol/l

*HMG-CoA reductase

**Triacylglycerols & fatty acids**

*TAG & FFA*

- 15 kg in nonobese subjects
- 570 000 kJ; enough for 3 months
- Thermal isolation, fertility, body shape
- Intake & synthesis: 80 – 170 mmol/d
- Different fatty acids – saturated, unsaturated, polyunsaturated (eikosanoids), shorter and longer chain
- Rapid turnover dependent on diet and alcohol intake, breakdown through physical activity (FFA – minutes)
3 lipoprotein families (LP)
- spherical or discoid particles synthesised in guts or liver
  - Hydrophilic surface (phospholipids, cholesterol esters and apoproteins)
  - Hydrophobic inner part (TAG, CH)
  - Dynamic interaction with vessel wall and each other
- Chylomicrons $\Rightarrow$ chylomicron remnants
- VLDL $\Rightarrow$ IDL $\Rightarrow$ LDL (heterogenous group*)
- HDL nascent $\Rightarrow$ HDL$_3$ $\Leftrightarrow$ HDL$_2$

*including “black sheep” Lp(a)

Apoproteins

<table>
<thead>
<tr>
<th>APOPROTEIN, FUNCTION</th>
<th>LOCATION OF THE GENES</th>
<th>MAIN OCCURRENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>A 1 – ACTIVATOR OF LCAT</td>
<td>11q23-qter</td>
<td>HDL, CHY</td>
</tr>
<tr>
<td>A 2 – ACTIVATOR OF LIVER LIPASE,</td>
<td>11q21-q23</td>
<td>HDL, CHY</td>
</tr>
<tr>
<td>INHIBITOR OF LCAT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A 4 – LCAT ACTIVATION</td>
<td>11q23-qter</td>
<td>HDL, CHY</td>
</tr>
<tr>
<td>B 100 LIGAND OF LDL RECEPTOR</td>
<td>2p23-24</td>
<td>LDL, IDL, VLDL</td>
</tr>
<tr>
<td>B 48 SHORT FORM OF B 100</td>
<td>ib.</td>
<td>CHY</td>
</tr>
<tr>
<td>C 1 – LCAT COFACTOR</td>
<td>19q12-q13.2</td>
<td>CHY, VLDL</td>
</tr>
<tr>
<td>C 2 – LPL ACTIVATOR (LIPOPROTEIN LIPASE)</td>
<td>19q12-q13.2</td>
<td>CHY, VLDL, HDL</td>
</tr>
<tr>
<td>C 3 – LPL &amp; LIVER LIPASE INHIBITOR</td>
<td>11q23-qter</td>
<td>CHY, VLDL, IDL</td>
</tr>
<tr>
<td>D – CHOLESTEROL ESTER TRANSFER REGULATION</td>
<td>3q14.2-qter</td>
<td>HDL</td>
</tr>
<tr>
<td>E – RECEPTOR LIGAND</td>
<td>19q12-q13.2</td>
<td>CHY, VLDL, IDL</td>
</tr>
</tbody>
</table>
And other genes...

<table>
<thead>
<tr>
<th>Protein</th>
<th>Location of the gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL receptor</td>
<td>19p13.2</td>
</tr>
<tr>
<td>HDL receptor</td>
<td></td>
</tr>
<tr>
<td>Lipoprotein lipase</td>
<td>8p22</td>
</tr>
<tr>
<td>Hepatic TG lipase</td>
<td>15q21</td>
</tr>
<tr>
<td>LCAT</td>
<td>16q12-q21</td>
</tr>
<tr>
<td>CETP</td>
<td>16q12-q21</td>
</tr>
<tr>
<td>Apo(a) for LP(a)</td>
<td>6q26-q27</td>
</tr>
</tbody>
</table>

Chylomicrons

- Enterocytes
- apoB48, others from HDL
- TAG into fat and other tissues, LPL
- CH into liver (from bile and food)
- Peak 3 – 6 h after meal, t₁/₂ 30 min, after 9 h Ø
- Remnants into liver through receptor cytotoxic and atherogenic
**VLDL – LDL family**

- Liver, endogeneous TAG, CH
- B100 and others
- functions and metabolism similar to CHY
- VLDL $t_{1/2}$ 2 – 4 hod, transformation to IDL, LDL
- **LDL has a slow turnover, can be modified – oxidation, glycation**
- small dense LDL
- Receptor and scavenger receptor
The reality is different

Site A: Lys Ala Gln Tyr Lys Lys Asn Lys His Arg His
Site B: Arg Leu Thr Arg Lys Arg Gly Leu Lys Leu Ala

B = binding site to LDL receptor

Without apoB the synthesis of LDL a CHY is not possible!
**Cholesterol = 6000**

**ApoB100 = 4**

**1500**

**Same cholesterol concentration**

**Different ApoB – higher in the case of small dense particles**

**LDL classes**

<table>
<thead>
<tr>
<th>LDL class</th>
<th>density, g/ml</th>
<th>diameter, nm</th>
<th>% of LDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1,025</td>
<td>27,5 – 26,0</td>
<td>3</td>
</tr>
<tr>
<td>II</td>
<td>1,028</td>
<td>26,0 – 25,5</td>
<td>16</td>
</tr>
<tr>
<td>III</td>
<td>1,034</td>
<td>25,5 – 24,2</td>
<td>50</td>
</tr>
<tr>
<td>IV</td>
<td>1,048</td>
<td>24,2 – 21,8</td>
<td>22</td>
</tr>
<tr>
<td>V</td>
<td>&gt; 1,048</td>
<td>&lt; 21,8</td>
<td>9</td>
</tr>
</tbody>
</table>
Reverse transport of cholesterol by HDL

- Liver, enterocytes and from CHY as nascent „disc”
- Lot of apoproteins
- LCAT and CEPT
  - lecithin-cholesterol acyltransferase, esterifies CH
  - cholesterol ester transfer protein transprots CH-E from HDL into other LPs
- Takes out cholesterol from tissues, disc is filled to HDL₃
- Exchanges CH-E for TAG with other LPs transforms to HDL₂
- Binds through AI to specific receptor in liver
What is the essence of the association of lipids and atherosclerosis???

- "Bad and good" lipids, lipoproteins
- Epidemiological studies – onlyz statistics: they are true but do not answer the basic question „why?“
- Atherosclerosis is the consequence of endothel dysfunction – inappropriate response to chronic injury
- Not only lipids

Endothel

- Intelligent interface between blood and vessel wall/tissues
- 1500 g, football field (1000 m²)
- Endocrine, paracrine and autocrine functions
  - vessel tonus, coagulation, adhesion, cell replication
- Organ specificity, differences in arteries, capillaries a venes
- Dysfunction in hypertension, diabetes, dyslipidemia...
Risk factors (CHD, IM)

- **Basic biological**
  - age, gender, family history (= genes)

- **Biochemical, classical**
  - cholesterol (§ 22), LDL-CH, TAG, ↓ HDL-CH, apoproteins, Lp(a), indexes

- **Biochemical, new**
  - fibrinogen, homocysteine, ferritin (Popeye)
  - small dense LDL, oxidized LDL

- **Nutrition and life style**
  - too much fat, (?) sugar, antioxidant and fiber deficiency
  - SMOKING, SEDENTARY LIFE STYLE

- **Diseases**
  - obesity, diabetes (IR!), hypertension, kidney failure

- **Genetic RF**
  - LDL receptor (FH), apo E variants and many others

Comments on Clinical Chemistry

- Total cholesterol – also after meal
- TAG – after 12h fasting
- HDL cholesterol – basic parameter
- LDL cholesterol – calculated ???
- LDL cholesterol direct !
- apoproteins, Lp(a) & other special assays
- indexes
Biochemical diagnosis of dyslipoproteinemia

- High number of CH a TAG assays
- Total cholesterol in healthy adults every 5 years
- Basic panel: TCH, HDL-CH, TAG
  - LDL calculated = TCH – (HDL-CH + TAG/2,2)
  - not possible if TAG > 4,5 mmol/l
- Direct LDL assay

Normal (desired) values

- T-CH < 5,0 mmol/l 4,55 – 5,45
- HDL-CH > 1,0 mmol/l 0,87 – 1,13
- LDL-CH < 3,0 mmol/l 2,65 – 3,35
- TAG < 2,0 mmol/l 1,70 – 2,30

INDEXES
- T-CH/HDL-CH < 5,0
- NONHDL-CH < 3,8
- LDL-CH/HDL-CH < 3,0
- Klimov (T-CH – HDL-CH)/HDL-CH < 4,2
- APO AI/APO B > 1,3
Other laboratory methods

- Special
  - Apo B, Apo AI, Lp(a)
  - homocysteine, coagulation and fibrinolytic factors.
- Very special
  - LDL receptors
  - Apo C, E, LPL, CETP, LCAT
  - classes of LDL, HDL
- Yesterday
  - total lipids, phospholipids, fatty acid esters
  - elektrophoresis
- Tomorrow - genomics

Classifications

- Fredrickson 1967, WHO 1970
  - I, IIa, IIb, III, IV, V, VI - partly history
- Therapeutical 1992, European task force
  - CH, TAG, both
- Etiological – future
  - primary and secondary DLP is not sufficient!
  - most primary DLP are not exactly characterized
“Secondary DLP”

- Nutrition and lifestyle
  - including smoking, alcohol and micronutrient deficiency
- Obesity
- Diabetes mellitus
  - type 2 usually, decompensated type 1 (BG 20 – extreme TAG)
- Kidney failure
- Liver disease
- Endocrine diseases – ↓ thyroid function
- Drugs
- Hormones – anticonception, gravidity, postmenopausal, anabolics

“Primary DLP”

- Familiar hypercholesterolemia (LDL rec.)
- Familiar defect of ApoB100 (FDB)
- ? Polygenous hypercholesterolemia
- ? Polygeneous hypertriglyceridemia
- ? Dysbetalipoproteinemia (IDL)
- ? Familiar type V hyperlipidemia
**E apoprotein genes and types**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Occurrence, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>e2/e2</td>
<td>&lt; 2</td>
</tr>
<tr>
<td>e3/e3</td>
<td>60</td>
</tr>
<tr>
<td>e4/e4</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>e2/e3</td>
<td>23</td>
</tr>
<tr>
<td>e2/e4</td>
<td>&lt; 2</td>
</tr>
<tr>
<td>e3/e4</td>
<td>12</td>
</tr>
</tbody>
</table>

E3 = 112 Cys, 158 Arg  
E2 = 112 Arg, 158 Arg  
E4 = 112 Cys, 158 Cys